

⑫ **EUROPEAN PATENT APPLICATION**

⑳ Application number: **86105801.4**

⑤① Int. Cl.⁴: **C 12 N 9/00, C 12 N 9/56,**
C 12 N 9/28

㉔ Date of filing: **26.04.86**

③① Priority: **06.05.85 US 730864**

⑦① Applicant: **MILES LABORATORIES, INC., 1127 Myrtle Street, Elkhart Indiana 46514 (US)**

④③ Date of publication of application: **12.11.86**
Bulletin 86/46

⑦② Inventor: **Brothers, Charles Everett, 25481 Jefferson Center Street, Cassopolis Michigan 49031 (US)**
Inventor: **Kim, Cohong Yol, 2623 California Road, Elkhart Indiana 46514 (US)**

⑧④ Designated Contracting States: **AT BE CH DE FR GB IT LI LU NL SE**

⑦④ Representative: **Dänner, Klaus, Dr. et al, c/o Bayer AG Konzernverwaltung RP Patentabteilung, D-5090 Leverkusen 1 Bayerwerk (DE)**

⑤④ **Process to solubilize enzymes and an enzyme liquid product produced thereby.**

⑤⑦ This invention relates to a novel process for the recovery of enzymes obtained from enzyme-producing microorganisms, and to the liquid enzyme product recovered by this process. Typically, the enzyme-containing filtrate from a fermentation of an enzyme-secreting microorganism is concentrated and a precipitation agent such as a salt or an organic solvent is added to the concentrate, thereby forming a cake. Then, a polyol solvent is circulated through the cake to solubilize the enzyme or enzyme complex from the cake and provide a liquid enzyme product. Particularly effective is propylene glycol as the polyol solvent. The liquid enzyme product may be shipped as is or subjected to further treatment to remove the solvent and create an essentially solvent-free enzyme product. The process is especially effective for the recovery of alkaline protease or alpha amylase.

- 1 -

PROCESS TO SOLUBILIZE ENZYMES
AND AN ENZYME LIQUID PRODUCT PRODUCED THEREBY

This invention relates to a novel process for the recovery of an enzyme liquid product. The enzymes contemplated are those provided by enzyme-producing microorganisms, whether intracellular or extracellular. More particularly, the invention contemplates solubilizing or dissolving a precipitated enzyme or enzyme complex in a polyol solvent. The invention is particularly effective for the recovery of alkaline protease or alpha amylase in a liquid product form.

BACKGROUND OF THE INVENTION

Enzymes behave as biocatalysts, regulating many of the chemical reactions that naturally occur in living organisms. When isolated, enzymes also have many industrial, as well as medical uses. For instance, enzymes are used in the tanning industry and the detergent industry. Moreover, enzymes have

many uses in the food industry, such as in the manufacture of cheese and alcoholic beverages.

In general, the traditional method in the production of enzymes has been to dissolve the enzyme in a water solution. Water, however, evaporates easily. Some enzymes, especially alkaline protease, are known to be potential health hazards to workers, and accordingly, it is desirable to keep them solubilized, i.e., prevent drying and/or dust formation. Dust and aerosols containing such enzymes can produce bronchial allergic reactions in sensitized persons. See, Flindt, "Pulmonary Disease Due to Inhalation of Derivatives of *Bacillus Subtilis* Containing Proteolytic Enzyme", The Lancet, from the Department of Occupational Health, University of Manchester, pages 1177-1184, (June 14, 1969). Moreover, enzymes such as alkaline protease (AP) easily precipitate out of a water solution. Thus, industrial production of such enzymes has been difficult due to their crystallization during the concentration steps employed in the traditional methods of production. These problems produced erratic yields and processing delays.

Thus, researchers had sought methods to keep enzymes, especially those that produce allergic reactions, dissolved in a closed system during processing. Nothing in the prior art, however teaches or suggests the use of a solvent other than

water (or water with minor additives) to solubilize precipitated enzymes.

The prior art discloses that organic solvents, such as propylene glycol (PG), ethylene glycol (EG), and polyethylene glycol (PEG), may be employed during enzyme preparation. For instance, U.S. Patent 4,497,897 discloses extraction of proteinase from Subtilisin Carlsberg using a solution of PG doped with carboxylate salt and calcium salt. U.S. Patent No. 3,242,056 discloses a process employing aliphatic polyols in the preparation of lysozyme to promote heat stability in the lysozyme final product. U.S. Patent No. 3,147,196 discloses a process in which tannin is added to an acidic enzyme-containing solution, and then the tannin-precipitated enzyme is extracted with an aqueous solution, which may contain PG or EG. However, tannin also ends up in the aqueous extract which is undesirable since tannin negatively interferes with the end use of the enzyme. Thus, additional processing is required so that the result is a solid, enzyme final product that is tannin-free. Also, U.S. Patent No. 3,440,143 discloses extracting enzymes from plant tissue with an aqueous solution containing 0.5-5% of a high molecular weight PEG having at least 25 ethylene units to precipitate the phenols naturally present in plant tissue. None of the literature, however, suggests or discloses the present novel

discovery of employing a novel solvent to prepare a solution of the enzyme.

SUMMARY OF THE INVENTION

5 The present invention provides for a process
for the recovery of an enzyme product wherein the
enzyme is provided by an enzyme- containing
solution obtained from an enzyme- producing
microorganism, said process comprising (a) adding a
precipitation agent to the enzyme-containing
10 solution to form a cake containing an enzyme or
enzyme complex which is essentially insoluble in
the solution and precipitates therefrom, (b)
separating the cake containing the enzyme or enzyme
complex from the solution, and (c) contacting the
15 cake with a polyol solvent to solubilize the enzyme
or enzyme complex from the cake to provide a polyol
solution of the enzyme or enzyme complex, whereby a
liquid enzyme product is recovered. Step (a) may
be optionally preceded by concentrating the enzyme-
20 containing solution, such as by evaporation or
ultrafiltration. Also, step (c), may be optionally
preceded by removing excess mother liquor to
provide a relatively drier cake containing the
enzyme or enzyme complex.

OBJECT AND ADVANTAGES

Accordingly, it is an object of the present invention to prepare commercially acceptable enzymes in a safer manner with good yield and
5 satisfactory purity. The invention affords several advantages. Not only do workers like the ease of handling a liquid product as opposed to the difficulty of handling dry enzyme solids, but also they like avoiding inhaling enzyme dust. For instance,
10 since polyols are hygroscopic and have a low vapor pressure, they do not evaporate as easily as water. Thus, a spill of a polyol solution of enzyme will not so readily produce enzyme dust if allowed to dry unnoticed as a water solution of enzyme would.

15 Furthermore, an ancillary advantage of the present invention is that polyols also have characteristics known to contribute to enhanced heat stability such as is disclosed in U.S. Patent No. 3,242,056 mentioned above.

20 Moreover, because undesired, inadvertent enzyme crystallization has been avoided, this new method has significant economic advantages, which can be seen from the Chart below. This Chart is intended for illustrative purposes only and is not
25 to be construed as required for teaching how to practice the present invention.

CHARTRECOVERY PROCESS FORALKALINE PROTEASE LIQUID PRODUCT

Comparison of Present Invention (1,2,3,4,5,6,7,8)

5 with Traditional Method (1,2,3,4,5A,6A,7A)

1. Fermentor
2. Drop tank
3. Drum filter
4. Ultrafiltration

10	5A. Evaporation: slow; high energy costs; enzyme crystalli- zation probable	5. Na_2SO_4 precipitation of AP-containing cake
	6A. Polishing: slow; possible loss of solid AP	6. Separating cake with plate frame filter*
15	7A. Formulation: relatively crude AP preparation since impurities have been concen- trated with AP	7. Extraction by recycling PG** through AP- containing cake collected on the plate frame filter
20		8. Formulation of PG extract***

* many impurities discarded in filtrate

** continuously ≥ 5 recycles; volume of PG is
25 about 1/10 of volume prior to precipitation in
No. 5 above

*** substantially purer enzyme product at higher
yield of AP

The Chart illustrates an embodiment of the present invention, wherein the enzyme alkaline protease (AP) is extracted with propylene glycol (PG), as compared to the traditional method of enzyme preparation. A culture of an enzyme-secreting microorganism is grown in a fermentor (Step 1). Then, the fermentation products are moved from the fermentor into the drop tank (Step 2). A flocculant may be added in the drop tank to aid in removing solids thereby producing an enzyme-containing solution that is run through a drum filter (Step 3). The enzyme-containing solution then may be concentrated, usually by a factor of 2 using ultrafiltration (Step 4). Next, Na_2SO_4 is added to precipitate a cake containing the enzyme (Step 5) and liquor is removed from the cake with a plate frame filter (Step 6). The liquor carries with it many impurities, such as colorants, odors, et cetera. In the present invention the enzyme is handled in a liquid phase, i.e., it is dissolved in a polyol solvent which is PG in this Diagram (Step 7). Thus, the present invention avoids the possibility of undesired crystal formation as in the traditional method (Step 6A). Accordingly, worker exposure is minimized since the enzyme is in an enclosed system (Step 7) essentially until final recovery. Depending on desired end use, the liquid PG extract of AP may be marketed as is, or formulated (Step 8),

which typically involves dilution with a compatible solvent such as water. Thus, from the Chart, it can be seen that the equipment, manpower and energy intensive steps of the traditional method are avoided by the present method. A substantially purer enzyme product results. It is now possible to obtain a liquid AP product with consistency and in good yield, which was not possible with traditional processing.

10 DETAILED DESCRIPTION OF THE INVENTION

 In general, the present invention will work with any enzyme provided by an enzyme-containing solution obtained from an enzyme-producing microorganism. The enzyme may be intracellular or extracellular. A solution of an intracellular enzyme may be obtained by any of various, known methods to rupture the cell membrane, such as using detergents, sonication, milling, grinding, osmotic pressure, lysis, and the like, to release the intracellular enzyme from the cells, followed by removal of the cell debris.

 Preferably, the enzyme is an extracellular enzyme provided by an enzyme-containing solution produced by the fermentation in a nutrient growth medium of enzyme-secreting microorganisms, such as bacteria, yeast, or fungi, followed by removal of the nutrient growth medium. The invention works

especially well with enzymes selected from pro-
teases, amylases, amyloglucosidases, lipases, and
oxidases. In the preferred embodiment, the ferment-
tation product, alkaline protease, is employed,
5 which is useful in several industries, particularly
the detergent industry.

After the microorganism produces the enzyme,
typical processing involves conventional methods,
such as filtration or centrifugation, to separate
10 the solids and/or cell debris from the solution
containing the enzyme. It is not necessary but it
is preferred at this point that this solution
containing the enzyme is then concentrated by at
least a factor of 2 by means such as ultrafil-
15 tration or evaporation.

Next, a precipitation agent, such as a salt or
a low molecular weight organic solvent is added to
the enzyme-containing solution, or in the preferred
embodiment where there has been concentration, then
20 to the concentrated solution. Addition of the
precipitation agent causes the enzyme and/or enzyme
complex to precipitate, and a "slurry" or "cake" is
produced. Throughout the description and claims,
the term "cake" may be used interchangeably with
25 the term "slurry", and it is intended to include
those instances where the "cake" is so wet that it
would be considered a "slurry". The cake contain-
ing the enzyme or enzyme complex is then separated
from the remaining solution. Usually this

separation is achieved by filtration and the filtrate containing impurities may be considered waste. If there is still excess mother liquor in the cake, it can be substantially removed from the slurry or cake by employing any of several methods. For instance, the excess mother liquor may be removed by additional regular filtration or by a pressure differential (such as suction filtration), gravity sedimentation, or centrifugation. The removal may be followed by a water wash and air blowing, providing a relatively drier cake.

The precipitation agents employed in the present invention are innocuous. By the term "innocuous" it is intended to mean that the precipitation agents contemplated by this invention (1) do not destroy the enzyme of interest, (2) do not negatively influence the end use of the enzyme product, and (3) do not require extensive additional processing to remove. It is unnecessary that the enzyme product be free of the precipitation agent. Thus, the precipitation agents contemplated by the present invention are other than those such as tannin, disclosed in the above-mentioned U.S. Patent 3,147,196. The presence of tannin in the enzyme product is very undesirable because tannin interferes with the availability of active enzyme sites. The precipitation agents contemplated by the present invention are broadly useful for many enzymes.

It is preferred to employ a salt as the precipitation agent in the present invention, but low molecular weight organic solvents will work well too as long as they are compatible with the particular polyol employed for solubilizing the enzyme. Preferred organic solvent precipitation agents are methyl ethyl ketone, acetone, methanol, ethanol, 1-propanol, isopropanol, tert-butanol, n-butanol, dimethyl formamide, dimethyl sulfoxide, monoethyl ether of ethylene glycol, monomethyl ether of ethyl glycol, and the like.

Organic solvent precipitation agents may be added to the solution containing the enzyme in a volume amount of 2 to 3 times the volume of the enzyme-containing solution. In a preferred embodiment with ethanol as the precipitation agent, the enzyme-containing solution is first concentrated by a factor of two and the volume of ethanol is 2.5 times the volume of the concentrated enzyme-containing solution.

If a salt is used as the precipitation agent, it should be selected from the Group I metal salts, the Group II metal salts, the corresponding ammonium salts of the Group I or II metal salts, or mixtures thereof. It is preferred that the valency of the anion of the salt be divalent or higher. Preferred are the phosphate, sulfate, and citrate salts. The especially preferred salts are sodium phosphate, ammonium phosphate, sodium citrate,

sodium sulfate and ammonium sulfate. Potassium and
cesium salts may also be employed, but of course
these are more expensive. Sulfate salts are most
desirable. Salt precipitation agents may simply be
5 added to the solution containing the enzyme, in the
amount of 5-50% weight/volume of salt agent to
enzyme-containing solution. More preferably, the
salt agent is added in the amount of 12-25%
weight/volume. Also, the salt agent may be dis-
10 solved in water and the aqueous solution added.

Next, a polyol solvent, which is PG in the
preferred embodiment, is circulated through the
cake in order to solubilize and recover the enzyme
and/or enzyme complex from the cake. It is
15 intended here that the term "to solubilize" means
the same thing as the term "to dissolve" or "to
extract" and the terms may be used interchangeably.
Also, the term "polyol solvent" as used here is
intended to mean 100% polyol, essentially 100%
20 polyol, or a polyol-containing solution wherein the
polyol is in combination with a compatible
co-solvent.

The polyols contemplated in this invention
comprise low molecular weight polyethylene glycol
25 and the C_2 through C_8 alcohols having at least two
OH groups. C_2 - C_8 alcohols with more than two OH
groups, such as glycerol, may be employed, but it
is preferred that there be present only two OH
groups. It is especially desirable that these two

OH groups be present on adjacent carbon atoms in the chain, and that the C_2-C_8 alcohol be aliphatic and have a straight carbon chain. Suitable polyols include, for example, ethylene glycol, propylene glycol, glycerol, the low molecular weight (about 5 900 or less) polyethylene glycols, and mixtures thereof.

The polyol may be in solution with a co-solvent for the enzyme, said co-solvent being 10 compatible with the polyol. The co-solvent of course may be water but also may be selected from organic solvents such as acetone, methyl ethyl ketone, methanol, ethanol, 1-propanol, isopropanol, tert-butanol, dimethyl formamide, dimethyl sulfoxide, 15 monomethyl ether of ethylene glycol, monoethyl ether of ethylene glycol, and the like. If the polyol is used in solution with a co-solvent, it is preferred that the polyol be present in an amount of at least 20% by volume, and more preferably 50%. 20 Higher concentrations of polyol, up to 100% polyol with no co-solvent, may also be advantageously employed. Also, the amount of co-solvent may depend on the co-solvent used. For instance, ethanol may also be used as a precipitation agent, i.e. in step 25 (a) of the Summary of Invention mentioned supra. Thus, too much ethanol as a co-solvent with the polyol may cause precipitation rather than solubilization of the enzyme.

The polyol solvent may be circulated through the enzyme-containing cake once, but preferably it is recirculated through the cake at least twice to enhance extraction of the enzyme. It is particularly desirable to employ at least 5 recirculations, and up to as many as 100, or more recirculations may be advantageously employed. The result is a liquid enzyme product, which is a polyol solution of the enzyme or enzyme complex. If a salt precipitation agent has been used, the resultant polyol solution of the enzyme or enzyme complex may be cooled to a temperature in a range between room temperature and the freezing point of this solution to cause excess salt to precipitate. In a preferred embodiment with alkaline protease, the cooling is down to approximately 16°C.

Depending on the desired end use, the polyol solution of the enzyme or enzyme complex may be used as is, as a liquid enzyme product, or the solvent may be substantially removed so that the enzyme by itself may be used. Removal of the solvent may be achieved by one or more known techniques or combinations thereof, thereby providing a substantially solvent-free enzyme product. One such technique is ultrafiltration, and another is reprecipitation of the enzyme followed by filtration and/or centrifugation to remove liquid.

The present invention also contemplates re-slurrying of the cake in the polyol solvent, but

more safety features result from recirculating the polyol solvent through the cake. Recirculation typically occurs in a closed system, i.e. the polyol solvent may be flowing through a pipe. On the other hand, when a cake is re-slurried, it is exposed to air and could become too dry, thereby subjecting the worker handling it to inhalation of enzyme dust. Nevertheless, an advantage of the present invention is that even if the closed system becomes exposed to the air, the chance of the cake drying unnoticed and producing dust is minimal since polyols are hygroscopic.

Depending on the enzyme, adjusting the pH toward the acid range during recirculation or reslurrying may enhance extraction. A minor amount of an acid such as acetic, sulfuric or hydrochloric may be advantageously employed for pH adjustment.

Any polyol extract may be formulated, if desired. A preferred method involves extraction with propylene glycol as the polyol solvent and then formulating the PG extract by diluting it with a co-solvent such as diluting it with water to 30% volume PG extract and 70% volume H_2O . Any of the other co-solvents mentioned above may also be employed in formulating the extract. The reason for formulating is to cut the enzyme activity down to whatever is desired depending on the end use of the liquid enzyme product. Care must be taken not to use too much co-solvent during the formulation

or the enzyme may precipitate instead of remaining in solution.

In another preferred embodiment, the volume of the enzyme-containing solution or concentrated
5 solution immediately before the step of adding the precipitation agent as compared to the volume of the polyol solvent that is circulated through the cake is in a ratio of approximately 30:1 to 2:1, and more preferably 10:1.

10 The following examples illustrate the preferred embodiments of the present invention, and are not intended to limit the claims to the embodiments disclosed in the examples. The examples illustrate preferred embodiments employing alkaline
15 protease and alpha-amylase both of which are fermentation products of Bacillus licheniformis.

Fermentation of Bacillus licheniformis
to Produce Alkaline Protease

Media suitable for the fermentation of alkaline
20 protease for a 1000 liter fermentor are as follows:

	Soy Media	50-100 kg
	Sodium Citrate	4-5 kg
	Calcium Chloride Dihydrate	4-5 kg
	A Starch	50-200 kg
5	Antifoam	235-280 ml
	α -amylase (TAKA-THERM® L-170) ¹	40-55 gm
	Mono- and Disodium phosphate	14-17 kg
	Water	added to 1000 L total volume

1. TAKA-THERM® is a trademark of Miles Laboratories, Elkhart, Indiana, for a broad class of carbohydrase enzymes. The particular TAKA-THERM used here is α -amylase.

The media was inoculated with viable cells of Bacillus licheniformis and allowed to ferment for 30 to 48 hours at 35-40°C. After this fermentation, the broth was diluted with H₂O by 50% of the initial drop volume and flocculated by a suitable flocculant to aid in biomass removal. The flocculated biomass was removed by centrifugation and the liquid passed through a precoated vacuum drum filter to provide a cell-free filtrate. The Detergent Alkaline Protease Units per milliliter (DAPU/ml) was determined by the Manual of Detergent Alkaline Protease Assay, and was between 60 and 70 DAPU/ml.

Fermentation of Bacillus licheniformis
to Produce Alpha-Amylase

Media suitable for the fermentation of alpha-amylase for a 1000 liter fermentor are as follows:

5	Sodium Citrate	0-5	kg
	Calcium Chloride		
	Dihydrate	0.2-1.0	kg
	Mono- and Dipotassium		
	Phosphate	15-24	kg
10	Ammonium Sulfate	2-7	kg
	A Sugar	100-200	kg
	Cotton Seed Meal)	25-40	kg
	Soy Media	30-50	kg
	Antifoam	8-13	L
15	Water	added to	1,000 L total volume

The media was inoculated with viable cells of Bacillus licheniformis and allowed to ferment for 70-90 hours at 40-45°C while maintaining the pH at approximately neutral. After this fermentation, the media was flocculated by a suitable flocculant to aid in biomass removal. The biomass was removed by centrifugation and the liquid passed through a drum filter to provide a cell-free filtrate. The Modified Wohlgemuth Units per milliliter (MWU/ml) was determined by the Manual Liquefying Alpha-Amylase Assay which is a modification of the

method disclosed by Wohlgemuth in Biochem. 29:1
(1908), and was between 100,000 and 120,000 MWU/ml.

EXAMPLE I

5 The filtrate (enzyme-containing solution) from
the 1000 liter alkaline protease fermentation was
concentrated by ultrafiltration through PM-2
membranes down to about 500 liters of concentrate.
The PM-2 membranes are polysulfone membranes
supplied by Romicon Company. The shortened nota-
10 tion PM-2 is used to indicate the membrane is
permeable by substances having a molecular weight
of approximately 2000 or less. Sodium sulfate was
then dissolved in the concentrate in the amount of
17% weight/volume, resulting in a slurry of enzyme
15 precipitate. FW-6 Dicalite™ admix (an inert
silaceous filter aid supplied by Eagle Pitcher
Industries) was added to the slurry in the amount
of 0.6% weight/volume of concentrate to enhance the
rate of filtration. The slurried batch was fil-
20 tered through a Sparkler™ apparatus. The Sparkler
filter apparatus is supplied by Sparkler Manufac-
turing Company of Conroe, Texas and some patents
covering these filter apparatus are U.S. Patent
2,460,423, U.S. Patent 2,760,641, and U.S. Patent
25 2,639,251. A Sparkler apparatus employs horizon-
tally disposed paper-type filters. Pressure was
applied to remove excess mother liquor and provide

about 25 kg of filter cake containing the alkaline protease precipitate. Next, the cake was washed with a minimal amount of water and then blown with ambient air to displace the balance of the mother liquor. Next, 20 liters of propylene glycol were recirculated through the cake inside of the filter apparatus for 2.5 hours in order to dissolve the enzyme. During recirculation, the pH of the PG solution of alkaline protease was periodically adjusted with acetic acid to 6.2 ± 0.2 . Afterward, cold water was then run through the filter apparatus jacket reducing the temperature of the propylene glycol solution of enzyme to approximately 16°C . The cooling caused precipitation of excess sodium sulfate, thereby removing this excess from the propylene glycol solution containing the enzyme. The result was an enzyme liquid product comprising a propylene glycol solution of alkaline protease. Recovery was calculated by assaying a small portion of the 500 liter concentrate and comparing that enzyme activity to the enzyme activity determined from assaying a small portion of the PG solution of the enzyme. Recovery of enzyme was 84%.

25

EXAMPLE II

The procedure of Example I was repeated, except that a plate-frame filter apparatus was

MS-1385

employed instead of a Sparkler™ filter apparatus.
A plate-frame filter apparatus employs vertically
disposed cloth-type filters. No water wash was done
in this apparatus. Recovery was calculated in the
5 same manner as Example I and was 80%.

EXAMPLE III

The procedure of Example II was repeated,
except that a filter-press apparatus was employed
instead of a plate-frame filter apparatus, and
10 after filtering the apparatus was cracked open
enough to allow excess mother liquor to drain from
the slurry, and then reclosed for extraction of the
enzyme with PG. Recovery was calculated in the
same manner as in Example I and was 74%.

15 EXAMPLE IV

The procedure of Example I was repeated,
except that 22% weight/volume of ammonium sulfate
was employed instead of the sodium sulfate.
Recovery was calculated in the same manner as
20 Example I and was 85%.

EXAMPLE V

The procedure of Example I was repeated except
that the filtrate from the 1000 liter alpha-amylase

fermentation was employed instead of the filtrate from the alkaline protease fermentation, and the amount of Na_2SO_4 was 22% weight/volume. Also, there was no pH adjustment with acetic acid.

5 Recovery was calculated in the same manner as Example I and was 84%.

EXAMPLE VI

10 The procedure of Example II was repeated except that the filtrate from the 1000 liter alpha-amylase fermentation was used instead of the filtrate from the alkaline protease fermentation and 22% weight/volume Na_2SO_4 was used. Also, there was no pH adjustment with acetic acid. Recovery was calculated in the same manner as Example I and
15 was 84%.

EXAMPLE VII

20 The procedure of Example III was repeated except that the filtrate from the 1000 liter alpha-amylase fermentation was used instead of the filtrate from the alkaline protease fermentation and the amount of Na_2SO_4 was 22% weight/volume. Also, there was no pH adjustment with acetic acid. Recovery was calculated in the same manner as Example I and was 84%.

EXAMPLES VIII - XV

The procedures of Examples I-VII, respectively, were repeated, except that instead of circulating the PG through the cake in the filter apparatus, the cake was removed from the filter apparatus and re-slurried in the PG. Recoveries were calculated in the same manner and were approximately 82% to 89%.

EXAMPLE XVI

The procedure of Example I was repeated, except that 1250 liters of ethanol were employed as the precipitation agent instead of the Na_2SO_4 , resulting in a slurry of an enzyme precipitate. Thus, it was unnecessary to cool to remove excess Na_2SO_4 . Also, the ethanol kept the extraction sufficiently acidic so that no pH adjustment with acid was necessary. Recovery was calculated in the same manner as in Example I and was 85%.

WE CLAIM:

1. A process for the recovery of an enzyme product wherein the enzyme is provided by an enzyme-containing solution obtained from an enzyme-producing microorganism, said process comprising;

- (a) adding an innocuous precipitation agent to the enzyme-containing solution to form a cake containing an enzyme or enzyme complex which is essentially insoluble in the solution and precipitates therefrom;
- (b) separating the cake containing the enzyme or enzyme complex from the solution; and,
- (c) contacting the cake with a polyol solvent to solubilize the enzyme or enzyme complex from the cake to provide a polyol solution of the enzyme or enzyme complex, whereby a liquid enzyme product is recovered.

2. The process of Claim 1, wherein the contacting with polyol solvent in step (c) is achieved by circulating a solution containing at least 20% by volume polyol in combination with a co-solvent for the enzyme at least once through the filter cake.

3. The process of Claims 1 or 2, wherein the contacting with polyol solvent in step (c) is achieved by re-slurrying the cake with a solution containing at least 20% by volume polyol in combination with a co-solvent for the enzyme.

4. The process of any of the Claims 1 to 3, wherein the co-solvent is acetone, methyl ethyl ketone, methanol, ethanol, 1-propanol, isopropanol, t-butanol, n-butanol, dimethyl formamide, dimethyl sulfoxide, monoethyl ether of ethylene glycol, monomethyl ether of ethylene glycol, water, or a mixture thereof.
5. The process of any of the Claims 1 to 4, wherein the contacting with polyol solvent in step (c) is with essentially 100% polyol.
6. The process of any of the Claims 1 to 5, further including (d) formulating the polyol solution of the enzyme or enzyme complex by dilution with water or an organic solvent on a volume/volume basis in the range of 99-30% polyol solution of enzyme or enzyme complex and 1-70% water or organic solvent.
7. The process of any of the Claims 1 to 6, wherein the enzyme-producing microorganism is Bacillus licheniformis.
8. The process of any of the Claims 1 to 7, wherein the enzyme is selected from the group consisting of proteases, amylases, amyloglucosidases, lipases, and oxidases.
9. The process of Claim 2, 3, or 5, wherein the polyol employed is a low molecular weight polyethylene glycol, a C₂-C₈ polyol, or a mixture thereof.

10. The process of Claim 9, wherein the polyol is glycerol, ethylene glycol, propylene glycol, polyethylene glycols having a low molecular weight of about 900 or less, or a mixture thereof.